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1. [6426190](#). 09 Aug 99; 30 Jul 02. Difference detection methods using matched multiple dyes.

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2. [6197501](#). 24 Jun 96; 06 Mar 01. Arrangement of nucleic acid sequences for comparative genomic hybridization. Cremer; Thomas, et al. 435/6; 435/5 435/91.2 536/23.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68 C12P019/34 C07H021/02.

3. [6127134](#). 20 Apr 95; 03 Oct 00. Difference gel electrophoresis using matched multiple dyes. Minden; Jonathan, et al. 435/7.2; 435/4 435/6 436/63 436/80 436/800 530/344 530/412. G01N033/53 G01N033/00 C12Q001/00 C07K001/00.

4. [6043025](#). 10 Oct 97; 28 Mar 00. Difference gel electrophoresis using matched multiple dyes. Minden; Jonathan, et al. 435/4; 204/459 204/461 435/810 436/172 436/63 436/800 436/86. C12Q001/00 G01N033/68.

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*09/06/94 701***WEST****Freeform Search**

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END OF SEARCH HISTORY

L3: Entry 1 of 7

File: USPT

Jul 31, 2001

DOCUMENT-IDENTIFIER: US 6268141 B1

TITLE: Immobilization of unmodified biopolymers to acyl fluoride activated substrates

Abstract Text (1):

A method of attaching unmodified biopolymers, particularly, unmodified polynucleotides, directly to a solid support is provided. The method includes the steps of (a) providing unmodified biopolymers; (b) providing a solid support having at least one surface comprising pendant acyl fluoride functionalities, and (c) contacting the unmodified biopolymers with the solid support under a condition sufficient for allowing the attachment of the biopolymers to the solid support. The unmodified biopolymers may be nucleic acids, polypeptides, proteins, carbohydrates, lipids and analogues thereof. The unmodified polynucleotides may be DNA, RNA or synthesized oligonucleotides. The DNA may be single or double stranded. A device including a solid support and unmodified biopolymers attached to the solid support by reaction with the pendant acyl fluoride functionalities of the solid support is also provided. The methods and devices of the present invention may be used in performing hybridization assays and immunoassays.

Brief Summary Text (5):

Biopolymer synthesis and biopolymer analysis often require the attachment of biopolymers to solid supports. For example, organic and inorganic materials have been utilized for the solid phase synthesis of peptides, oligonucleotides and small organic molecules. The synthesis involves the stepwise addition of activated monomers such as amino acid derivatives or nucleotide derivatives to a growing oligomeric chain attached at one end to a solid support. At the completion of the synthesis, the newly synthesized biopolymers may be cleaved from the solid support and subsequently utilized in biochemical research or diagnostic applications or, alternatively, be utilized without cleaving the biopolymers from the solid support.

Brief Summary Text (6):

For biopolymer analysis, biopolymers may be attached to a solid support in several ways. In blotting techniques, native biopolymers are first captured onto a membrane and subsequently immobilized on the membrane by heat, radiation or chemical techniques. The immobilized biopolymers are then available for subsequent analyses, such as those associated with southern blotting applications and reverse hybridization analytical techniques.

Brief Summary Text (8):

It is generally understood that reactive groups present within native polynucleotides are weak and therefore make for inefficient attachment. In addition, when native polynucleotides are exposed to highly reactive surface groups, excessive crosslinking may occur. This crosslink may prevent the attached nucleic acid from fully participating in hybridization. These conditions are most noticeable for short fragments of double-stranded DNA or oligonucleotides. Thus, oligonucleotides often have to be modified, for example, derivatized to a 5'-amino terminus, for an effective attachment. The 5' amino-linker allows selective binding of the amino-containing DNA to silylated slides through a Schiff's base reaction with aldehyde groups on the chip surface. The selectivity of amino-modified versus natural, unmodified DNA is about 10:1 for cDNAs and about 10,100:1 for single-stranded 15-mers. DNA molecules of intermediate lengths exhibit intermediate discrimination ratios. In addition, the 5' end attachment of the DNA to the chip via the amino group permits steric accessibility of the bound molecules during the hybridization reaction. Therefore, post-modification has been perceived as obligatory for attachment of, e.g., oligonucleotide probes for creation of arrays. Such post-modification processes require additional time-consuming steps at substantial costs.

Brief Summary Text (9):

Therefore, it is desirable to develop a more effective method for attaching biopolymers, particularly unmodified biopolymers to a solid support. It is particularly desirable to develop a method to directly attach unmodified biopolymers, such as polynucleotides, to a solid support.

Brief Summary Text (11):

The present invention is based on the discovery that both short and long fragments of single-stranded or double-stranded DNA may be efficiently attached to acyl fluoride activated supports directly without spacer arms and without modifying the polynucleotide. It is also based on the discovery that other biopolymers, such as protein A, antibodies, streptavidin, ect., may also be attached to a solid support without modifications to the biopolymers.

Brief Summary Text (21):

(d) contacting the solid support attached with the unmodified agent with the biopolymer target, or contacting the solid support with the attached, unmodified biopolymer target with the agent under a condition that allows the formation of a complex comprising the agent and the biopolymer target;

Brief Summary Text (23):

A further aspect of the present invention provides a device comprising a plurality of unmodified biopolymer and a solid support. The solid support has at least one surface comprising pendant acyl fluoride functionalities, and the biopolymer is attached to the solid support by reaction with the pendant acyl fluoride functionalities.

Detailed Description Text (8):

It is a discovery of the present invention that biopolymers of the present invention may be attached to a solid support without any modification to the biopolymers. For example, in accordance with one embodiment of the present invention, biopolymers such as, but not limited to, polynucleotides, protein A, antibodies or streptavidin may be attached to a solid support without any modifications to the biopolymers.

Detailed Description Text (11):

Briefly, polymeric materials suitable for fabricating solid supports can be any material capable of being derivatized to form acyl fluoride functionalities on at least one surface of the solid support. For example, polymeric materials with pendant carboxyl functionalities or polymeric materials capable of being modified to support carboxyl groups can be reacted with suitable reagents to form acyl fluoride functionalities. In one embodiment of the present invention, solid supports are fabricated of ethylene acrylic acid copolymers, ethylene methacrylic acid copolymers, or derivatized polypropylene. Those skilled in the art will recognize that polymeric materials capable of being derivatized to support carboxyl groups which in turn can be modified to provide surface acid fluoride functionalities include a wide range of materials. For example, aminated polypropylene reacted with a cyclic anhydride, e.g., succinic anhydride, to provide carboxyl groups suitable for converting to acyl fluoride is particularly useful.

Detailed Description Text (20):

Because the solid support of the present invention is particularly useful in the preparation of biopolymer arrays for the evaluation or identification of biological activity, the solid support is preferably in the form of a device having at least one flat planar surface. The size of the solid support can vary and depends upon the final use of the immobilized biopolymers. Those skilled in the art will appreciate that arrays of biopolymers immobilized on miniaturized solid supports have been under development for many years. These solid supports can be measured in terms of mm.sup.2 and can have numerous different immobilized biopolymers, each with different biopolymers attached to a different site specific location on the miniaturized solid support. Solid supports in the form of dip sticks are also within the scope of the present invention. As known in the art, dip sticks typically are rectangular in shape with each side measuring a few centimeters. On the other hand, large biopolymers such as polynucleotide arrays, utilized for sequencing whole genomes, may have dimensions measuring a meter or more.

Detailed Description Text (23):

The biopolymers of the present invention are attached to a solid support of the present invention by contacting the unmodified biopolymers with the solid support under a condition sufficient for allowing the attachment of the biopolymers to the solid support. A condition is sufficient if it allows the unmodified biopolymers to react with acyl fluoride of a solid support for covalently attaching the biopolymers to the solid support. While not wanting to be bound by the theory, it is believed that, under the conditions of the present invention, unmodified biopolymers may be attached to a solid support by displacement of the fluoride group contained in the solid support with a nucleophile of a biopolymer.

Detailed Description Text (28):

In accordance with embodiments of the present invention, the concentration of unmodified biopolymers contained in aqueous solutions may vary, depending on the type of molecule, the molecule size, the molecule structure, and other factors that may influence solubility of the molecules. For example, when the attached polymers are polynucleotides, preferably they are in the range of 5 nM to 40:M. More preferably, they are in the range of 5 nM to 5:M.

Detailed Description Text (31):

In accordance with one embodiment of the present invention, at least 1 to about 1536 different unmodified biopolymers may be attached to at least 1 to about 1536 discrete isolated areas in a solid support of the present invention

Detailed Description Text (32):

Attachment of unmodified biopolymers to acyl fluoride activated solid supports is well-suited for use in the construction of genosensors and other array-based systems such as differential gene expression micro-arrays. A solid support with attached unmodified biopolymers of the present invention may also be used as a device for performing a ligand binding assay or for performing a hybridization assay by either reverse hybridization (probes attached) or southern blot (target attached). Such a device may also be used in an immunoassay.

Detailed Description Text (37):

(d) contacting the solid support attached with the unmodified agent with the biopolymer target, or contacting the solid support with the attached, unmodified biopolymer target with the agent under a condition that allows the formation of a complex comprising the agent and the biopolymer target;

Detailed Description Text (43):

Another aspect of the present invention also provides a device for performing hybridization assays, immunoassay or other assays. A device of the present invention comprises a plurality of unmodified biopolymers and a solid support. The solid support has at least one surface comprising pendant acyl fluoride functionalities, and the biopolymer is attached to the solid support by reaction with the pendant acyl fluoride functionalities.

Detailed Description Text (44):

For the purpose of the present invention, the attached biopolymers may be the same or different. If the biopolymers are different, preferably they are located in discrete, isolated areas of the solid support to form arrays. For example, a solid support may be a microplate. Different biopolymers may be attached to different wells of the microplate for forming arrays. In accordance with one embodiment of the present invention, at least 1 to 1536 unmodified biopolymers, such as probes, may be attached to at least 1 to 1536 wells of a microplate.

Detailed Description Text (45):

The solid support of the device, such as a microplate, may be surface treated with acyl fluoride functionalities, and then biopolymers may be attached to the solid support by reaction with the pendant acyl fluoride functionalities. Alternatively, biopolymers of the present invention may be printed onto the surface of a plastic disk containing pendant acyl fluoride functionalities, and the disk is then inserted into the bottom of the microplate well.

Detailed Description Text (53):

Biotips with cDNA arrays attached were denatured for 15 minutes in 200 .mu.L

denaturant (0.15 M NaCl, 0.5M NaOH), then rinsed in stringency buffer (2.times.SSC, 0.01% SDS, pH 7.0) just prior to hybridization. In the case of Primer Array Biotips, no denaturation step was used. Biotin-labeled PCR products of actin, G3PDH, p53 and TNF alpha derived from a 1.sup.st strand cDNA pool (liver) were prepared for hybridization to the Primer Arrays or cDNA Arrays as follows: 10 .mu.L PCR solution was diluted with 10 .mu.L water and 50 .mu.L denaturant added. The solution was incubated for 10 minutes at ambient temperature followed by the addition of 150 .mu.L of neutralization buffer (0.3M Tris, pH 7.5, 2.4.times.SSC, 0.02% SDS). After mixing, the solution was placed in well of a 24-well polypropylene cell culture plate and the Biotip immersed. Hybridization was allowed to proceed for 60 minutes at 25.degree. C. for cDNA Arrays of 60.degree. C., 60 minutes for Primer Arrays with shaking in a humidified chamber. The Biotips were then removed from the hybridization solution and placed in another well containing 2.times.SSC, 0.01% SDS for a stringency rinse (at the same temperature used for hybridization) for 10 minutes. Upon a final rinse in the above stringency buffer, the Biotip was blotted to remove excess solution and placed in streptavidin-alkaline phosphatase conjugate for 30 minutes at ambient temperature. Following extensive rinsing in stringency buffer, the Biotip was again blotted, then placed in ELF reagent (fluorescent substrate for alkaline phosphatase, Molecular Probes, Inc.) for signal development. The signal was allowed to develop for 30 minutes. Following a brief water rinse, the array signal was read using a CCD camera system.

Detailed Description Text (60):

After spotting, the arrays were incubated at 25.degree. C., 1 hour in a humidified chamber. They were then removed from the chamber and allowed to air-dry 25 minutes at ambient temperature, followed by an additional drying at 30.degree. C. for 10 minutes. The plastic supports were then immersed in ethanol for 60 minutes to block residual reactive surface groups, followed by a final 10-minute soak in deionized water.

Detailed Description Text (76):

Polypropylene film was surface aminated by radio frequency plasma (U.S. Pat. No. 5,554,501) and amine functionalities converted to carboxyl groups by reaction with succinic anhydride. The acyl fluoride activation was accomplished using the DAST reagent as described previously in the copending U.S patent application Ser. No. 08/797,222. Acyl fluoride activated polypropylene film was stored dry under argon at -20 C until needed.

Detailed Description Text (78):

Unmodified cDNA from pcr amplification of .sub.1 st strand cDNA (liver tumor) was purified by gel filtration spin column (XTreme, Pierce Chemical) to remove primers, dNTPs, cofactors and Taq enzyme. The purified cDNA was eluted from the columns with water, then diluted into 0.5 M sodium bicarbonate buffer, pH 9 for coupling. A Biomek 2000.TM. robotic system equipped with a 384-pin HDRT system was used to print cDNA solutions onto the substrate. A set of BAPA markers, 5-(Biotinamido)pentylamine (Pierce Chemical), were also printed at both ends of the film, thereby flanking the tethered cDNAs. BAPA, which binds streptavidin-enzyme conjugate independently of hybridization, serves as an internal control for assay robustness. Following printing, the films were dried at 35 C for 15 minutes and then immersed in ethanol for two hours to block residual surface reactive groups. The films were briefly rinsed in deionized water and allowed to air dry. The 8 cm.times.12 cm film was sectioned into 12 pieces with each strip containing two copies of a 3.times.3 replicate of each cDNA along with six copies of 3.times.3 replicates of the BAPA marker. The resulting cDNA Array film strips were stored dry at -20.degree. C. or room temperature prior to hybridization.

Detailed Description Text (99):

Proteins (Protein A, streptavidin, rabbit anti-goat IgG) were diluted into 1 M carbonate buffer, pH 9 or 10, to a concentration of 0.5-1.0 mg/mL. The protein solution was applied to the surface of acyl fluoride activated plastic Biotips in 0.5 .mu.L drops. The tips were incubated in a high humidity chamber at 25.degree. C. for 1 hr. They were then removed from the chamber and allowed to air dry completely (.about.15 min) before being placed in the quenching/blocking solution. The tips were quenched by placing in 1.0 mL solution in a 24-well plate and vortexing vigorously for a minimum of 30 min. After quenching, the tips were rinsed with deionized water for 10 min with shaking and air dried.

L3: Entry 2 of 7

File: USPT

Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6146833 A

TITLE: Polymeric reagents for immobilizing biopolymers

Abstract Text (1):

Reagents for the immobilization of biopolymers, processes for their preparation and their subsequent use in the immobilization of biopolymers for analytical and diagnostic procedures are described. One type of reagent includes a solid support fabricated of a polymeric material having at least one surface with pendant acyl fluoride functionalities. Another reagent includes solid supports fabricated of polymeric materials including ethylene acrylic acid or ethylene methacrylic acid copolymers and activated polypropylene. Processes for preparing reagents include derivatizing polymeric materials to form acyl fluoride functionalities or derivatizing ethylene acrylic acid copolymers and ethylene methacrylic acid copolymers to form active acyl functionalities. Processes for immobilizing biopolymers include attaching natural or presynthesized biopolymers to activated solid support surfaces and directly attaching in a step-wise successive manner biomonomer units to a growing biopolymer chain attached to the solid support reagent.

Brief Summary Text (5):

For many years solid phase chemistry has found a rapidly expanding utility in biopolymer synthesis and biopolymer immobilization. For example, organic and inorganic materials have been utilized for the solid phase synthesis of peptides and oligonucleotides by the step wise addition of activated amino acid derivatives or nucleotide derivatives to a growing oligomeric chain attached at one end to a solid support. Typically, the solid support material is in the form of a porous bead or resin material having a high surface area. Typically, in these solid phase systems, the chemically synthesized peptides or oligonucleotides are cleaved from the solid support and subsequently utilized in biochemical research or diagnostic applications. Thus, the solid support material plays no role in the research or diagnostic applications, having been used only during the initial synthesis of the biopolymer.

Brief Summary Text (9):

Derivatized polypropylene films, glass slides and silicon wafers have been used for the solid support synthesis of oligonucleotides and peptides at site specific locations on the film, slide or wafer. These materials have been fairly successful because the glass, polypropylene and silicon withstand the physical and chemical rigors of the synthesis and hybridization processes. Furthermore, these materials are suitable when fluorescence detection techniques are used because they have low background fluorescence. However, glass slides, silicon wafers and polymer films are difficult to handle, and require handles or specially designed holders in order to manipulate the solid support when they are utilized in automated processes. These handles or holders are expensive to design and use and generally add to the costs of the utilizing solid supports. Furthermore, it is difficult to characterize and control the surface density of biopolymers synthesized or attached to glass slides and polypropylene films. Thus, there is a continuing need for improved materials suitable for immobilizing biopolymers and for materials suitable for directly synthesizing biopolymers. Such materials are preferably also suitable as solid supports for the evaluation of immobilized biopolymer biological activity, their identification or their use in analytical applications.

Brief Summary Text (21):

In another aspect, the present invention provides processes for preparing an immobilized biopolymer which include first providing a solid support fabricated of a polymeric material and having at least one surface incorporating pendant acyl fluoride functionalities. Then contacting the surface with derivatized biopolymer or biomonomer under appropriate reaction conditions results in the attachment of the derivatized

biopolymer or biomonomer to the solid support surface. Typically, when the solid surface is utilized to immobilize biomonomer the process further includes successively reacting biomonomer units to form a growing biopolymer which is attached or immobilized to the solid support surface. In preferred embodiments the solid support is fabricated of ethylene acrylic acid copolymer or ethylene methacrylic acid copolymer which has been derivatized to form pendant fluoride functionalities.

Detailed Description Text (3):

More particularly, a first aspect of the present invention includes reagents for immobilizing a biopolymer, the reagent including a solid support fabricated of a polymeric material having at least one surface with pendant acyl fluoride functionalities. Because the reagents of the present invention are particularly useful in the preparation of biopolymer arrays for the evaluation or identification of biological activity, the solid support is preferably in the form of a device having at least one flat planar surface. The size of the solid support can vary and depends upon the final use of the immobilized biopolymer. Those skilled in the art will appreciate that arrays of biopolymers immobilized on miniaturized solid supports have been under development for many years. These solid supports can be measured in terms of mm.sup.2 and can have numerous different immobilized biopolymers with each different biopolymer attached to a different site specific location on the miniaturized solid support. Solid supports in the form of dip sticks are also within the scope of the present invention. As known in the art dip sticks typically are rectangular in shape with each side measuring a few centimeters. On the other hand, large biopolymer arrays such as oligonucleotide arrays utilized for sequencing whole genomes may have dimensions measuring a meter or more.

Detailed Description Text (6):

Those skilled in the art will recognize that polymeric materials capable of being derivatized to support carboxyl groups which in turn can be modified to provide surface acid fluoride functionalities include a wide range of materials. For example, aminated polypropylene reacted with a cyclic anhydride, e.g. succinic anhydride, to provide carboxyl groups suitable for converting to acyl fluoride is particularly useful. Another suitable polymeric material includes polyvinyl alcohol derivatized with, for example, an alkyl diacid to form a pendant carboxyl group: ##STR1## Additional suitable polymeric materials include methylmethacrylate or methylacrylate saponified to expose a pendant carboxyl group: ##STR2## Still other polymeric materials easily derivatized to provide a reactive carboxyl group are hydrolyzed polyacrylonitrile or hydrolyzed polymethacrylonitrile: ##STR3##

Detailed Description Text (11):

In preferred processes, the derivatized biopolymers are amino derivatized biopolymers, e.g. amino derivatized oligonucleotides and peptides having the following general structures: ##STR7## The amino derivatized oligonucleotide shown is derivatized at its 5' end phosphate. However, those skilled in the art will appreciate that any number of sites on the oligonucleotide can be selected to attach the amino group, including the base sites on the sugar moieties. Amino derivatized biopolymers are preferred because amide linkages resulting from the reaction of acyl fluoride functionalities with the amino functionalities are stable and the reaction kinetics associated with the amide bond formation is favorable. However, it is also within the scope of this aspect of the present invention to provide processes for immobilizing biopolymers which involve contacting suitably derivatized biopolymers with the acyl fluorides under conditions which result in ester bond formations. Biopolymers suitable in the practice of this embodiment include alkyl hydroxy or hydroxy derivatized oligonucleotides and peptides. Those skilled in the art will recognize that reaction conditions can be tailored so that a nucleotide phosphate hydroxyl reacts with acyl fluorides to immobilize an oligonucleotide.

Detailed Description Text (16):

While the reagents of the present invention are particularly useful for immobilizing presynthesized or available oligonucleotides, proteins and peptides, they are also suitable for the direct solid support synthesis of oligonucleotides and peptides. Thus, the present invention additionally provides processes for attaching a biomonomer unit to a solid support fabricated of a suitable polymeric material and the subsequent stepwise successive addition of biomonomer units to a growing biopolymer chain. In accordance with this aspect, preferred embodiments involve directly synthesizing

oligonucleotides or peptides to ethylene acrylic acid copolymers or ethylene methacrylic acid copolymers which have been derivatized to provide a "linker" compound attached via an amide moiety to the copolymer. Advantageously, the amide moiety is stable to the chemistries used during the synthesis procedures and can be formed by directly reacting an amine with the carboxyl functionality of the copolymer. Alternatively, the process can involve forming an amide linked "linker" compound by reacting an amine with an acyl halide functionality formed on the surface. The acyl halide can be acyl fluoride prepared as described above or any other acyl halide prepared utilizing methods known in the art.

Detailed Description Text (22):

Polypropylene film (purchased from Mobil) having a thickness of approximately 0.03 mm was aminated by exposing the film surface to a radio frequency plasma in the presence of ammonia following the procedures described in U.S. Pat. No. 5,112,736. The aminated film was then washed with acetonitrile and then derivatized to form carboxyl functionalities linked to its surface by contacting the aminated surface with an aqueous solution of 0.1 M NaHCO<sub>3</sub> and 0.1 M succinic anhydride in a closed container for about 16 hours. The film was removed from the aqueous solution and washed once with methanol followed by three washes with isopropyl alcohol and air drying.

Detailed Description Text (24):

The carboxyl linked and amine blocked polypropylene film was then placed in a sealed container containing a 5 vol % solution of (diethylamino)sulfur trifluoride (DAST) in methylene chloride and shaken for about 16 hours. After removing the solid support from the reaction container it was washed with dichloromethane followed by an acetonitrile wash and air drying. This acyl fluoride derivatized film was stable during a six month storage under cool and dry conditions.

Detailed Description Text (28):

Ethylene acrylic acid copolymer, available from Dow Chemical Co. under the tradename Primacor, and ethylene methacrylic acid copolymer, available from DuPont under the tradename Nucrel, were injection molded into strips about 0.5 mm thick. Selected Nucrel strips were impregnated with carbon black during the molding process. Ethylene methacrylic acid copolymer film about 0.05 mm thick was obtained from DuPont. The molded strip and film was washed with acetonitrile and allowed to air dry. The dried strips and film were placed in a closed container containing a 5% v/v solution of (diethylaminosulphur)trifluoride in methylene chloride and shaken for about 16 hours. These acyl fluoride activated molded strips were then removed from the methylene chloride solution, successively washed with methylene chloride and acetonitrile and three times with acetonitrile and then air dried.

Detailed Description Text (54):

To derivatize the copolymer, a film of ethylene acrylic acid copolymer obtained from Banner packaging was suspended in anhydrous diethyl ether containing greater than 5 fold excess of phosphorous. After approximately 16 hours at room temperature the film was removed from the phosphorous pentachloride mixture and washed with anhydrous diethyl ether and dichloromethane. This acyl chloride activated copolymer was then placed in a saturated solution of 1,12 diaminododecane in dichloromethane for about 16 hours. This reaction provides an amino terminated twelve methylene linker attached to the solid support with an amide attachment. It was determined that the density of amino groups on the film surface was approximately 100 .mu.moles/cm.<sup>2</sup> which is more than 100.times. that of prior art plasma aminated polypropylene film.

Detailed Description Text (58):

The following describes the direct oligonucleotide synthesis onto a particulate solid support reagent of the present invention. Ethylene acrylic acid copolymer pellets purchased from Dow Chemical (Primacor #3460) were ground to a powder in a Waring blender. Five grams of the powder were placed in anhydrous diethylether containing an excess of phosphorous pentachloride. After 16 hours the copolymer powder was removed and washed with anhydrous diethyl ether and dichloromethane. This acid chloride derivatized powder was then placed in a saturated solution of 1,12 diaminododecane in dichloromethane for about 16 hours. This resulted in a particulate solid support synthesis reagent having an amino terminus and a twelve methylene length linker group. The linker is attached to the particulate polymer via an amide moiety.

Detailed Description Text (59) :

The amino terminated particulate copolymer was then reacted with [(pentachlorophenyl)succinyl]nucleoside (DMT-T-CO(CH<sub>2</sub>.sub.2).sub.2 -O-pcp) in pyridine and triethylamine for 2 days. This was followed by washing with pyridine, dichloromethane and pyridine and then air drying. This prepared solid support reagent was then sealed in a synthesis column sized for an Oligo 1000 automated DNA synthesizer (available from Beckman Instrument, Fullerton, Calif.). One of the cystic fibrosis oligonucleotides synthesized in Example 7 was synthesized according to standard phosphoramidite procedures provided by the manufacturer. During the synthesis normal dimethoxytrityl monitoring was observed following each deprotection step, indicating that normal synthesis was taking place.

Detailed Description Text (61) :

The following demonstrate the direct synthesis of peptides onto a synthesis reagent of the present invention. Pellets of ethylene acrylic acid copolymer (Primacor #3460) were flattened into the shape of small disks about 5 mm in diameter. These disks were washed with methanol and dichloromethane and then suspended in anhydrous diethyl ether containing an approximately five fold excess of phosphorous pentachloride. After about 16 hours at room temperature the disks were removed from the phosphorous pentachloride mixture and washed with anhydrous diethyl ether and dichloromethane. The acyl chloride activated copolymer disks were then placed in a saturated solution of 1,12 diaminododecane in dichloromethane for about 16 hours. This reaction provides an amino terminated twelve methylene linker attached to the solid support disks with an amide attachment.

Detailed Description Text (63) :

This prepared ethylene acrylic acid copolymer solid support was then utilized to attach a glycine residue followed by synthesizing the CRISPP P12 epitope sequence by conventional Fmoc/t-Bu procedures. During the synthesis Kaiser ninhydrin tests showed coupled and N-terminally deprotected activity through the synthesis. Following trifluoroacetic acid (TFA) side-chain deprotection with 0.5 mL ethanedithiol scavenger and 9.5 mL TFA at room temperature for 5 hours and a standard workup, the synthesized peptide was specifically detected by immunoassay procedures with a horseradish peroxidase developer.

Detailed Description Text (67) :

A 8.times.4 cm 0.5 mm thick strip of polypropylene film which had been previously plasma aminated as described above was washed 3 times with methanol, 3 times with acetonitrile and 3 times with isopropyl alcohol for 5 minutes each. After air drying, the strip was placed in an aqueous solution of 0.1 M NaHCO<sub>3</sub> and 0.1 M succinic anhydride. The solution and strip were shaken for about 16 hours and then washed with methanol and 3 times with isopropyl alcohol. After air drying, the strip was carboxylated by immersing it in 20 mL of acetic anhydride containing about 50 mg DMAP and shaking for 1 hour at room temperature.

## CLAIMS:

11. The process of claim 10, where the polymeric material provided is aminated polypropylene, and where derivatizing the polymeric material to form carboxyl functionalities comprises reacting the aminated polypropylene with an anhydride.

L3: Entry 5 of 7

File: USPT

Dec 10, 1996

DOCUMENT-IDENTIFIER: US 5583211 A

TITLE: Surface activated organic polymers useful for location - specific attachment of nucleic acids, peptides, proteins and oligosaccharides

Abstract Text (1):

Disclosed herein are surface activated, organic polymers useful for biopolymer synthesis. Most preferably, aminated polypropylene is used for the synthesis of oligonucleotides thereto, and these devices are most preferably utilized for genetic analysis of patient samples.

Brief Summary Text (5):

Deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA") are long, threadlike macromolecules, DNA comprising a chain of deoxyribonucleotides, and RNA comprising a chain of ribonucleotides. A nucleotide consists of a nucleoside and one or more phosphate groups; a nucleoside consists of a nitrogenous base linked to a pentose sugar. Typically, the phosphate group is attached to the fifth-carbon ("C-5") hydroxyl group ("OH") of the pentose sugar; however, it can also be attached to the third-carbon hydroxyl group ("C-3 OH"). In a molecule of DNA, the pentose sugar is deoxyribose, while in a molecule of RNA, the pentose sugar is ribose. The nitrogenous bases in DNA are adenine ("A"), cytosine ("C"), guanine ("G"), and thymine ("T"). These bases are the same for RNA, except that uracil ("U") replaces thymine. Accordingly, the major nucleosides of DNA, collectively referred to as "deoxynucleosides" are as follows: deoxyadenosine ("dA"); deoxycytidine ("dC"); deoxyguanosine ("dG"); and thymidine ("T"). The corresponding ribonucleosides are designated as "A"; "C"; "G"; and "U". (By convention, and because there is no corresponding thymidine ribonucleoside, deoxythymidine is typically designated as "T"; for consistency purposes, however, thymidine will be designated as "dT" throughout this disclosure).

Brief Summary Text (21):

Preferably, the external chemical species is: (1) a chemical species not previously adsorbed to the surface of the polymer; and (2) becomes a nucleophile when adsorbed to the surface of the polymer, preferably via an elevated energy state. Most preferably, the elevated energy state is a radio-frequency plasma discharge, a microwave frequency plasma discharge, or a corona discharge.

Brief Summary Text (23):

In a particularly preferred embodiment of the invention, the organic polymer is polypropylene, the external chemical species are nitrogen and hydrogen (in the form of an ammonia gas), the nucleophile is an amine, and the elevated energy state is achieved via radio frequency plasma discharge. Thus, a particularly preferred surface activated organic polymer is a polypropylene material aminated via radio frequency plasma discharge; such materials are preferably utilized for the "in-place", or "in-situ", attachment of nucleotides and/or amino acids thereto, without the need for spacer arms or linkers, and thus particularly well suited for the synthesis of oligonucleotides and/or peptides. The amine groups on the activated organic polymers are reactive with nucleotides such that the nucleotides and/or amino acids introduced thereto are covalently attached onto the surface of the polymer.

Drawing Description Text (2):

FIG. 1 is the result of High Pressure Liquid Chromatographic analysis of a cleaved 17-mer oligonucleotide (with 5'-DMT protecting group) previously synthesized directly onto aminated polypropylene;

Drawing Description Text (4):

FIG. 3 is the compilation of dehybridization analyses of three oligonucleotide probes

from a defined Target Oligonucleotide, the Target Oligonucleotide having been synthesized directly onto aminated polypropylene, each of the probes having various sequences which are complementary or non-complementary to the Target;

Drawing Description Text (5):

FIG. 4 is the compilation of dehybridization analyses of amplicons from CTFR Exon 10, Normal and CTFR Exon 10, .DELTA.F508, against Target Oligonucleotide complementary to CTFR Exon 10, Normal, the Target Oligonucleotide having been synthesized directly onto aminated polypropylene; and

Drawing Description Text (6):

FIG. 5 is a laser-printer reproduction of a hybridization between a fluorescent-labelled probe and a target synthesized directly onto aminated polypropylene, with detection of the label via a CCD camera.

Detailed Description Text (2):

The solid phase synthesis of biopolymers (e.g., oligonucleotides, peptides, oligosaccharides, lipids, etc.) requires, by definition, a solid support material from which the initial starting material is attached and from which the synthesis of the biopolymer is initiated. As has been noted, "The unique feature of solid phase synthesis is the solid support itself, and future improvement of the synthesis will probably depend upon finding better supports". Wallace, R. B. and Itakura, K. "Solid phase synthesis and biological applications of polydeoxyribonucleotides" Chpt. 13, Solid Phase Biochemistry Scouting, W. H., Ed. John Wiley & Sons (1983). As those in the art will appreciate, this statement has proven to be correct. As the chemistry involved in the synthesis of biopolymers has improved; as the need for such biopolymers, particularly oligonucleotides and peptides, has increased; and as the area of application of such biopolymers has expanded, the need for "better supports" has indeed increased.

Detailed Description Text (3):

In essence, the benefits associated with previous solid support strategies are typically also directly related to the types and number of problems occasioned by the use thereof. This is because to the degree that such materials are conducive to chemical interactions between the support material itself and the biopolymer, by that same degree the materials can interact in a non-specific manner with other materials. For example, nylon-based filters find wide-spread application in the area of DNA analysis whereby oligonucleotides are cross-linked directly to the nylon. However, nylon is very reactive with other materials such that it is typically necessary, if not essential, to chemically block any site on the nylon to which an investigator does not wish to have such "non-specific" materials bind thereto. As noted in the above-referenced Zhung article, in an effort to attempt to prevent the non-specific attachment of sample DNA to the location on the nylon membranes where amino-linker bound oligonucleotide probes were not located, it was necessary to block these highly reactive sites to avoid non-specific binding thereto. This protocol was examined in an effort to improve upon a previous procedure utilizing poly-T tails as a linker between a solid support and oligonucleotides. See, Saiki, R. K., et al (1989) PNAS USA 86:6230-6234. It is noted that in these procedures, the oligonucleotides are described as being synthesized "off-line" and subsequently attached to the support via an amino-linker or a poly-T tail.

Detailed Description Text (4):

In the case of "on-line" (in-situ) synthesis, the types of chemical manipulations that are required to be utilized in conjunction with the available solid supports are staggering. For example, in the case of inorganic solid supports, such as CPG, silica, glass, etc., the chemical structure of these materials creates a "rigidity" which is believed to constrain the synthesis of biopolymers. Thus, particularly in the case of CPG, it is typically necessary to utilize chemical linkers in conjunction therewith. In essence, these linkers, regardless of their chemical composition or length, are intended to provide a degree of "freedom" in the synthesis by providing a chemically "flexible" moiety which is attached to the support at one end and is capable of binding to the growing biopolymer. Without the linker, one can theoretically synthesize biopolymers, but overall yields, the rapidity of synthesis, etc. are jeopardized. As a further example of the use of inorganic materials which utilize linkers, European Patent Application No. WO 89/10977 discloses the use of glass plates

having aliphatic linkers bound thereto for use in the in-situ synthesis of oligonucleotides thereon.

Detailed Description Text (12) :

As used herein, the term "surface activated" when used in conjunction with polymer is intended to mean the process of modifying a polymer such that external chemical species become adsorbed onto the surface of the polymer, whereby the chemical species are capable of chemically linking biopolymers and biomonomers to the surface of the polymer. Preferably, the chemical linking is via a nucleophile, and most preferably the nucleophile is on the surface of the modified polymer.

Detailed Description Text (14) :

As used herein, the phrase "device-medium" when used in conjunction with the term "polymer" is intended to mean any device to which a polymer medium can be affixed such as, microtiter plates, test tubes, inorganic sheets, dipsticks, etc. For example, when the polymer medium is a polypropylene thread, one or more polypropylene threads can be affixed to a plastic dipstick-type device, or polypropylene membranes can be affixed to glass slides. The particular device is, in and of itself, unimportant--all that is necessary is that the polymer medium can be affixed thereto without affecting the functional behavior of the polymer or any biopolymer adsorbed thereon, and that the device intent is stable within any materials to which the device is introduced (e.g., clinical samples, etc.).

Detailed Description Text (15) :

As used herein, the term "adsorbed" is intended to have a meaning ordinarily ascribed thereto in the chemical and biochemical arts. Stated again, a first material that is adsorbed onto the surface of another material becomes, in effect, a "part" of that material such that the first material is not capable of being easily removed from the surface of the other material. For example, a surface activated polymer comprises nucleophiles on the surface thereof; under appropriate conditions, biomonomers, e.g., that react with the nucleophiles will be covalently attached, and therefore adsorbed, to the surface of the biopolymer via such nucleophiles.

Detailed Description Text (17) :

As used herein, the term "nucleophile" is a chemical species comprising a pair of electrons which are capable of combining with an electron-deficient species. Preferably, the external chemical species becomes a nucleophile when adsorbed to the surface of the polymer. An "external chemical species" is a chemical species not previously adsorbed to the surface of the polymer. Stated again, an external chemical species becomes a nucleophile when it is adsorbed to the surface of the polymer. Preferably, an external chemical species is amenable to a plasma process. Typically, the plasma process will create ionized and radical forms of the external chemical species. Preferably, the external chemical species are selected from the group consisting of: nitrogen; oxygen; sulfur; carbon; hydrogen; argon; helium; and combinations comprising at least one of the foregoing. Nucleophilic forms of these external chemical species include, e.g., amine; hydroxyl; thiol; carboxylate; and substituents comprising at least one of the foregoing. When an external chemical species is subjected to a plasma, ionized and radical form(s) of the external chemical species result. As those in the art appreciate, under appropriate conditions, such ionized and radical form(s) can "chemically" interact with the polymer, whereas the non-ionized and non-radical form(s) thereof do not have a tendency to form chemical bonds with the polymer.

Detailed Description Text (18) :

As used herein, the term "biopolymer" is intended to mean repeating units of biological or chemical moieties. Representative biopolymers include, but are not limited to, nucleic acids, oligonucleotides, amino acids, proteins, peptides, hormones, oligosaccharides, lipids, glycolipids, lipopolysaccharides, phospholipids, synthetic analogues of the foregoing, including, but not limited to, inverted nucleotides, peptide nucleic acids, Meta-DNA, and combinations of the above. "Biopolymer synthesis" is intended to encompass the synthetic production, both organic and inorganic, of a biopolymer. Related to a biopolymer is a "biomonomer" which is intended to mean a single unit of biopolymer, or a single unit which is not part of a biopolymer. Thus, for example, a nucleotide is a biomonomer within an oligonucleotide biopolymer, and an amino acid is a biomonomer within a protein or peptide biopolymer;

avidin, biotin, antibodies, antibody fragments, etc., for example, are also biomonomers. Additionally, as used herein, the term "initiation biomonomer" or "initiator biomonomer" is meant to indicate the first biomonomer which is covalently attached via reactive nucleophiles to the surface of the polymer, or the first biomonomer which is attached to a linker or spacer arm attached to the polymer, the linker or spacer arm being attached to the polymer via reactive nucleophiles.

Detailed Description Text (20):

As used herein, the phrase "reverse dot blot" is meant to indicate a protocol whereby biopolymers are attached to a solid support, and the presence (or absence) of constituents in a sample material are detected via the application, and subsequent interaction (or non-interaction), of the sample to the biopolymers. As used herein, the phrase "dot blot" is meant to indicate a protocol whereby constituents in or from a sample material are attached to a solid support and biopolymer or biomonomer probes are applied thereto.

Detailed Description Text (24):

As used herein, the term "seed" is meant to indicate a cell which binds to a bioreactive peptide and to which other similar cell may attach.

Detailed Description Text (29):

Polypropylene can be surface activated via the introduction of amino groups thereto using RFPD, MFPD or CD in ammonia gas or other suitable amine introducing entities including, but not limited, to C<sub>sub</sub>1 - C<sub>sub</sub>12 aliphatic or cyclic amines which may be primary, secondary or tertiary. The hydrocarbon chain can be straight chain, branched, saturated or unsaturated, and one or more amino groups can be attached to the hydrocarbon chain. Methyl amine, alkylamine, ethylenediamine, diaminocyclohexane are examples of such amines. Ammonia is most preferred.

Detailed Description Text (30):

In the presence of a RFPD, MFPD or CD, the most probable mechanism for the attachment of amino groups to a medium is as follows: ##STR4## In the presence of oxygen radical, the resulting surface activated polypropylene comprises the following activated surface: ##STR5## In the presence of sulfur radical, the resulting surface activated polypropylene comprises the following activated surface: ##STR6## Thus, for example, using well known nucleic acid synthesis techniques, one can obtain the following adsorbed-initiation nucleotides: ##STR7## where "A" is a protecting group and "B" represents one of the four bases. Because a chemical linker is not required to attach the biomonomer to the polymer, the initiation biomonomer is adsorbed, i.e., becomes, in effect, a "part" of the polymer itself.

Detailed Description Text (38):

Dissection of the polymer medium also allows for the generation of multiple media, each comprising the defined biopolymer. These can then be dissected and one piece from each medium can be affixed to a unitary device-medium. Thus, for example, a plethora of dipsticks can be readily created, each dipstick comprising several different polymer media sections comprising specific biopolymers; thus, the conditions for synthesis of any particular biopolymer is identical and such biopolymer is amenable to QA/QC protocols.

Detailed Description Text (40):

The disclosed polymers are particularly well suited for the direct synthesis of, e.g., oligonucleotides and peptides thereon. Beneficially, a variety of commercially available nucleic acid synthesizers are available, including the Beckman Instrument OLIGO 1000. Focusing on aminated polypropylene membranes or threads, immediately following the amination process (or after removal from storage), the materials can be incorporated directly into the reaction chamber of the nucleic acid synthesizer; because of the versatility of such materials, they can be readily manipulated within the chambers, i.e. loosely "rolled" in the case of membranes, or loosely inserted in the case of threads. Beneficially, synthesis of oligonucleotides can proceed directly onto the aminated polypropylene, and, owing to the "activated" nature of the amine groups, these oligonucleotides are covalently attached directly to the polypropylene ("cleavable" links or spacer arms can, of course, be utilized such that the oligonucleotides are amenable to removal; active esters of succinate nucleosides are preferred as these are susceptible to "cleavage" by ammonia).

Detailed Description Text (41):

Such derived oligonucleotides are ideally suited for utilization in genetic screening analyses. I.e., by utilizing aminated polypropylene comprising oligonucleotides, where the oligonucleotides are complementary to either the wild-type or mutation(s) sequence of a gene of interest, prepared patient samples can be screened for the presence or absence of the sequence of interest. It is preferred that the length of oligonucleotides chemically synthesized for use in such genetic analysis be up to about 250 bases in length, preferably between about 5 and about 100 bases, more preferably between about 8 and 30 bases, and most preferably about 16 bases. These lengths are to be construed as relative to the conditions under which the genetic analysis is conducted. For example, at room temperature (at which temperature we prefer to conduct the analysis) the most preferred length is 16 bases; at lower temperatures, shorter (i.e. 8-mers) can be utilized.

Detailed Description Text (44):

As will be further appreciated by the skilled artisan, because the DNA sample, by definition, comprising two complementary strands of DNA, amplification techniques such as PCR will generate sets of complementary amplicons (an "amplicon" is one set of strands of the amplification product, i.e. complementary "amplicons" are the resulting product of a PCR amplification). A suggested approach for increasing sample binding to oligonucleotides covalently attached to aminated polypropylene is to remove one of these sets from the reaction mixture prior to analysis; this has the effect of decreasing competition for hybridization of the complementary amplicons to each other. For this approach, only one of the amplicon sets is subjected to screening with the aminated polypropylene-oligonucleotide device. One approach to segregation of the amplicon sets involves manipulating the primers. For example, one set of primers can be biotinylated, and the other set can be, e.g., labelled for detection. Thus, after amplification, the biotinylated amplicons can be "removed" from the sample using, e.g., avidin-coated beads. This has the effect of maintaining substantially only labelled amplicons in the solution. The label, of course, can be utilized for detection purposes after the labelled amplicons are "screened" with the aminated polypropylene-oligonucleotide device.

Detailed Description Text (45):

Following such amplification, the sample material can be presented to the aminated polypropylene-oligonucleotide device which comprises an oligonucleotide having a sequence complementary to the labelled amplicons. Because a preferred application of the aminated polypropylene-oligonucleotide devices is screening genomic samples for genetic mutations, the stringency conditions (i.e. the conditions which allow hybridization and dehybridization to occur, including but not limited to: temperature, ionic strength, chemical conditions, time, and the nature of the sequences) are important. I.e., for mutations including only one change in nucleic acid sequences (vis-a-vis the wild-type, or "normal" sequence), it is very likely that in screening for a mutational sequence, even the normal sequence will readily hybridize to the complementary oligonucleotide. Thus, removal of such "non-specific" hybridization is essential. For example, this can be accomplished using a series of washings with decreasing salt concentrations, which has the effect of essentially removing substantially all of the non-specifically hybridized materials before removing substantially all of the specifically hybridized materials.

Detailed Description Text (46):

Because of the criticality of ensuring that only complementary sequences are detected, it is our current preference that an "historical" de-hybridization analysis be conducted so that we can "track" the dehybridization of the sample nucleic acid sequences from the aminated polypropylene-oligonucleotide device over time and stringency conditions--by comparing the dehybridization patterns of non-complementary and complementary sequences as they are removed from the aminated polypropylene-oligonucleotide device, we have ascertained that the patterns are substantially different such that an accurate assessment of "correct" hybridizations can be conducted. This historical analysis can be utilized for creating a "yes/no" stringency protocol for specific genetic analysis. For example, if a specific known mutation provides a specific known "historical" de-hybridization pattern, then if this pattern is obtained for an unknown sample, that sample comprises that specific mutation.

Detailed Description Text (47) :

In essence, we subject the hybridized material to a decreasing salt gradient using high performance liquid chromatography ("HPLC") techniques, while continuously monitoring the loss of signal (indicative of de-hybridization) over time. As will be set forth in detail below, we have experimentally determined that distinct differences in such de-hybridization patterns can be ascertained, such that accurate determinations as to the presence or absence of defined sequences can be made. For the analysis of genomic DNA using oligonucleotides which are synthesized directly onto, e.g., aminated polypropylene, such analysis may be readily conducted as indicated above.

Detailed Description Text (50) :

Alternately, one can synthesize different biopolymers onto different polymer mediums and combine these onto a single polymer device. For example, in the case of membranes, various sections from different polymer media comprising different oligonucleotides can be affixed to a single device. The benefit of this approach is that one can more readily quality control a portion of the polymer medium comprising the biopolymer such that the quality of a device comprising a portion of that polymer medium can be assured. In the case of cystic fibrosis, e.g. 95 separate polymer media (or a sub-population thereof) comprising 95 different oligonucleotides can be utilized, with each being dissected and portions from each being subjected to quality control procedures. Thereafter, these 95 media can be combined onto one (or several) unitary device medium.

Detailed Description Text (52) :

The disclosed surface activated polymers are particularly suited for *in situ* biopolymer synthesis for "reverse dot blot" protocols. For example, in certain of the protocols described above, a series of oligonucleotides having different defined sequences are synthesized directly onto a surface activated polymer (or synthesized "off-line" and attached to the polymer). Thereafter, a sample suspected of containing a polynucleotide having a sequence complementary to one of the oligonucleotides is applied thereto. Detection can be accomplished via a labelling scheme that provides for labelling of the polynucleotide prior to analysis (direct labelling) or after hybridization (indirect labelling). Because the investigator knows the physical location of the different oligonucleotides on the polymer medium, the presence of a label at a specific location provides information as to both the presence and the sequence of any material from the sample that has hybridized to the aminated polypropylene oligonucleotide device.

Detailed Description Text (55) :

Beneficially, a protein to be sequenced can be covalently attached to a surface activated, chemically inert organic polymer using, e.g., 1,4 phenylene diisothiocyanate ("PDITC"); however, any moiety capable of binding to the surface of the polymer and a protein can be utilized. The coupling reaction can proceed as follows: ##STR8## where: "PP-Nu" is polypropylene comprising a nucleophile adsorbed on the surface thereof.

Detailed Description Text (56) :

The protein, being covalently attached to the polymer, can then be efficiently sequenced using, e.g., Edman degradation protocols.

Detailed Description Text (57) :

Another application for the disclosed surface activated, chemically inert organic polymers can be directed to the covalent attachment of biomonomers such as avidin, avidin derivatives, biotin, and biotin derivatives. Such materials have a plethora of applications--particularly preferred is sequestering biotinylated or avidin-linked macromolecules, respectively. As those in the art appreciate, avidin is a glycoprotein having four binding sites specific for biotin; the binding affinity between biotin and avidin is both very strong and non-covalent. The utility of avidin, for example, attached to such a polymer is varied--as an example, such a material can be utilized to standardize the resulting sequence reaction of the amplification of a polynucleotide containing sample using, e.g., the PCR. For example, biotinylated primers (specific to a first region of a target sequence) are added to a polymer device medium having avidin covalently coupled thereto; thereafter, second, labelled

primers (specific to a second region of the target), polymerase enzyme, and nucleotide triphosphates are added to the bound primer and the PCR reaction is initiated. By controlling the amount of avidin attached to the polymer, the amount of biotinylated primer is "controlled", such that elongated, labelled strands which result form the PCR reaction are standardized, and these can then be analyzed utilizing, e.g., conventional slab gel electrophoresis techniques.

Detailed Description Text (58):

The surface activated, organic polymers can also be utilized for cell adhesion and cell growth/propagation for example, in devices used for mammalian cell culture, artificial skin grafts and prosthetic devices exhibiting tissue and blood compatibility. For example, bioreactive peptides directed to specific cell recognition domains can be synthesized onto the disclosed polymers, and samples comprising a variety of cells can be applied thereto, whereby those cells comprising the recognition domains can selectively attach thereto. Yamada, K. M., "Adhesion Recognition Peptides," J. Bio. Chem. 266:20 12809-12812 (1991), describes a variety of bioreactive peptides and the cells comprising recognition domains specific for such domains. For example, the adhesive glycoprotein fibronectin is involved in a variety of biological processes, particularly in mediating cell attachment and cell migration. Fibronectin is bound by several cell surface receptors; peptide sequences of fibronectin which are recognized by such receptors include Arg-Gly-Asp ("RGD") and Leu-Asp-Val ("LDV"). Thus, a series of biopolymers comprising one or more RGD peptides can be synthesized onto the disclosed polymers to, e.g., mediate cell attachment thereto. For example, Pierschbacher and Ruoslahti, Nature 309:30-33, 1984 (and cited references), first isolated and characterized a peptic digest fragment of fibronectin containing the cell attachment domain. A 30-amino acid synthetic peptide was prepared which carried the cell attachment promoting activity. The domain was further delineated to a tetrapeptide (RGDS) that promoted the attachment of rat kidney fibroblasts when attached via a 6-carbon atom spacer arm to Sepharose beads but not when the RGDS tetrapeptide was coupled to protein-coated plastic plates. The authors suggested that this lack of activity might be due to a decrease in accessibility of cells to the attachment domain or because of poor coupling efficiency to the protein-coated plastic surface. To alleviate this problem, Cappello and Crissman, Polymer Preprints 31: 193-194, 1990, utilizing recombinant genetics, inserted a 10-amino acid sequence of fibronectin that contained the RGD domain into a segment of the amino acid sequence encoding the crystalline region of the Bombyx mori silk fibroin protein. A high molecular weight copolymer SLP-F containing repeat sequences of the RGD recombinant peptide was immobilized onto nitrocellulose filters and promoted the attachment of african green monkey kidney epithelial cells. The supports disclosed herein can be utilized in an efficient manner to achieve these types of end-results.

Detailed Description Text (87):

Qualitative analysis of aminated polypropylene was conducted by ninhydrin analysis; ninhydrin, a heterocyclic compound, complexes with amine groups and during the complexation process, a color change, from yellow to a deep blue, occurs. Approximately 1-3 drops of each of the following solutions were added serially to putative aminated polypropylene material: A - potassium cyanide/pyridine (0.01M KCN/98ml pyridine); B - 500 mg ninhydrin/10 ml butanol; C - 80 mg phenol/20 ml butanol. This was followed by heating of the materials at 110.degree. C. for about 2 min, followed by qualitative observation of color.

Detailed Description Text (89):

Quantitative determination of amination of polypropylene was accomplished using a modification of the procedure set forth in Reddy, M.P. et al. "An efficient procedure for the solid phase tritylation of nucleosides and nucleotides." Tetrahedron Letters 28/1: 23-26 (1987). Amine groups react with dimethoxytrityl (DMT) chloride in the presence of tetra-n-butylammonium perchlorate/2,4, 6-collidine in dichloromethane; upon acid treatment, DMT cation is released and can be measured spectrophotometrically at 501 nm. Results can be presented as OD units/cm.sup.2 of aminated polypropylene.

Detailed Description Text (90):

The protocol was as follows: aminated polypropylene was suspended in equimolar solution (0.5M) of dimethoxytrityl chloride (Aldrich, St. Louis, Mo.) and tetra-n-butylammonium perchlorate (Fluka, City, State) in dry dichloromethane

containing 2,4,6-collidine. The reaction was completed within between about 15 to 30 minutes. The aminated polypropylene was removed and washed thoroughly with dichloromethane. Dry aminated polypropylene was thereafter suspended in 10 ml of 2% trichloroacetic acid in dichloromethane (w/v); the presence of an orange color indicated the presence of DMT cation, and this was quantified spectrophotometrically (.lambda.max - 501 nm, .epsilon.=76,000).

Detailed Description Text (96):

Synthesis of oligonucleotides was performed on a Beckman Instruments, Inc. (Fullerton, Calif.) OLIGO 1000 automated DNA synthesizer using phosphoramidite-based chemistry protocol. Aminated polypropylene was utilized for the solid support material. Homo- and hetero-oligonucleotides of various lengths were synthesized in accordance with manufacturer instructions.

Detailed Description Text (102):

In order to analyze the progressions of probe-target disassociation over time, a breadboard Dynamic Hybridization Analysis (D-HAS.TM.) System was constructed. For the D-HAS.TM. analyzer used herein, a Beckman Instruments, Inc. System Gold.TM. HPLC Programmable Solvent Module 126 equipped with a modified 171 radioisotope detector was utilized; the modification consisted of replacing the flow cell with a 1/8 inch o.d./1/16 inch i.d. fluorinated ethylene propylene copolymer tubing. This tubing allowed for insertion of aminated polypropylene-oligonucleotides having labelled sequences of interest hybridized thereto therein, and this material was in turn "sandwiched" between 2 polypropylene screens. This arrangement allowed for the flow of Disassociation Buffer through the modified flow cell--thus, as disassociation of the labelled sequence from the aminated polypropylene oligonucleotide occurred, the number of radioactive counts decreased, thus providing a continuous tracking of the disassociation of the probe from the target. Bottle A contained D-HAS.TM. gradient buffer and Bottle B contained 0.01% SDS. The D-HAS.TM. System was operated in a gradient mode as follows: 0-2 min - 100% Bottle A (1 ml/min); 2-22min - 0%-100% Bottle B (2 ml/min); 22-24 min - 100% Bottle B (2 ml/min); 24-26 min - 0%-100% A (2 ml/min).

Detailed Description Text (108):

The 3' end of Target A was directly synthesized to the aminated polypropylene (i.e. no "linker" was utilized).

Detailed Description Text (123):

The 3' end of Target A70 was directly synthesized to the aminated polypropylene.

Detailed Description Text (136):

The 3' end was directly synthesized to aminated polypropylene film.

Detailed Description Text (142):

Polypropylene membrane filter sheets and films were subjected to the RF amination procedure as delineated above. Following plasma amination, approximately 1 cm.times.1 cm edge cuts of the sheets and films were qualitatively analyzed for amine content by the ninhydrin reaction: non-aminated ("virgin") polypropylene control sheets and films evidenced no color change (i.e. the color remained yellow); aminated polypropylene sheets evidenced a blue color, indicative of amine groups present on the polypropylene sheets. The remainder of the sheets and films were stored at room temperature (in dark) in polyethylene bags, heat sealed using an electric wire impulse heat sealer. Prior to oligonucleotide synthesis, sheets and films were quantitatively analyzed for amine content by Sulfo-SDTB: virgin polypropylene evidenced about 0 to 1 nmoles/cm.sup.2 ; polypropylene membrane subject to RF treatment was determined to comprise between about 5 to 30 nmoles/cm.sup.2 .

Detailed Description Text (144):

Masking of polypropylene sheets (21.5 cm.times.26.6 cm) was accomplished by placing such sheets on the glass plate within the RF instrument and overlaying the sheet with a 30 cm.times.30 cm polypropylene mesh filter screen (Spectra/Mesh Los Angeles, Calif., Prod. No. 146410, 1000 .mu.m.times.1000 .mu.m nominal mesh opening). Plasma amination was conducted as delineated above. Qualitative testing for amine "patterning" (i.e. the presence of amine groups corresponding to the nominal mesh openings) was conducted using Sulfo-SDTB reagent, followed by rinsing in distilled water, followed by holding the sheets over fumes of concentrated hydrochloric acid. A

"checkered" pattern resulted where the unmasked areas had an orange color (indicative of the presence of amine groups) while the areas beneath the mesh portion (masked area) was white (indicative of the absence of amine groups). The same sheet was then rinsed in distilled water, methanol and acetone, followed by air drying. Thereafter, a 30 dyne-cm blue dye wetting Tension Test System Kit No. 5 (Select Industrial Systems, Waukesha, Wis.) was applied to the polypropylene sheet; the area covered by the mesh adsorbed blue dye, indicating that this area was hydrophobic (i.e. amine groups absent), while the unmasked areas remained white in appearance, indicating that this area was hydrophilic (i.e. amine groups present).

Detailed Description Text (146):

Determination of Oligonucleotide Presence on Aminated Polypropylene

Detailed Description Text (147):

Determination of the efficiency of oligonucleotide synthesis onto aminated polypropylene of Example I was predicated upon HPLC and CZE analysis of oligonucleotides synthesized onto the material and cleaved therefrom.

Detailed Description Text (150):

Prior to oligonucleotide synthesis, and owing to the objective of analyzing the synthesized material, a linker group was added to the aminated polypropylene. Specifically, the aminated polypropylene was condensed with the active ester of nucleoside succinate; this was followed by the addition of other nucleosides. As is appreciate, the succinate portion is amenable to "cleavage" using ammonia.

Detailed Description Text (151):

17-mer oligonucleotides (SEQ ID NO. 11) were synthesized directly onto aminated polypropylene sheets (0.5 cm.times.1.5 cm) which were hand-rolled and loosely packed into a needle-tip reaction column of a Beckman Instruments OLIGO 1000 DNA Synthesizer; aminated polypropylene threads (about 20 cm in length) were similarly utilized. Following synthesis of the 17-mer oligonucleotides, the oligonucleotides were cleaved from the supports with NH<sub>sub</sub>4 OH(28%) for 1 hr. at room temperature, followed by deprotection with NH<sub>sub</sub>4 OH(28%) for 1 hr. at 80.degree. C. Released oligonucleotides were then analyzed by HPLC and CGE techniques under the parameters set forth above. Results were presented in FIG. 1 (HPLC) and FIG. 2 (CGE) for oligonucleotides synthesized onto polypropylene sheets (results for polypropylene threads yielded substantially identical results; these results are not provided herein).

Detailed Description Text (152):

As is evident from FIGS. 1 and 2, a single, well defined peak is set forth, indicating, inter alia, that synthesis efficiency on the aminated polypropylene was optimal (the small peak in FIG. 1 is attributed to benzamide formed by the removal of benzoyl protecting groups on the synthetic DNA strand).

Detailed Description Text (155):

In order to determine the hybridization characteristics of polynucleotides to target sequences synthesized onto aminated polypropylene, a series of experiments were performed using oligonucleotides which had sequences which were perfect complements (i.e. analogous to "wild-type") and sequences which were not perfect complements (i.e. analogous to "mutations"). As noted, in genetic analysis, it is critical to differentiate between wild-type and mutation sequence; this goal is exacerbated given the ability of deleterious mutations to be considered by a single base deletion/substitution and the preponderance of such a mutated sequence to hybridize to a wild-type complement probe. Because of these factors, dehybridization from the target was analyzed over time using the D-HAS.TM. System.

Detailed Description Text (156):

Previously referenced Target A was directly synthesized onto aminated polypropylene membranes using the aforementioned DNA synthesizer. Thereafter, P23 (perfect compliment to Target A); P24 (single base mismatch); and P37 (two-base deletion) were separately introduced to Target A as follows: aminated polypropylene having Target A covalently bound thereto was equilibrated in Hybridization Buffer; thereafter, each of the three probes (0.5 pmoles/50 .mu.l Hybridization Buffer) were added to these membranes followed by 1 hour incubation at 25.degree. C. Hybridization Buffer was then

removed, and membranes rinsed once with 200 .mu.l Hybridization Buffer; thereafter, membranes were added to the D-HAS.TM. System as disclosed. Analysis of decreasing presence of labelled probes was conducted, with results being collectively presented in FIG. 3.

Detailed Description Text (158) :

These results indicate, inter alia, that wild-type target and mutation target(s) can be synthesized directly to aminated polypropylene and these can be used for genetic screening--the ability to differentiate between the presence of wild-type complement and mutation (or vice-versa) is evident.

Detailed Description Text (162) :

Focusing on CFTR Exon 10, the underlined portion of that Sequence (SEQ ID NO. 7) is referred to herein as the "regional mutation," i.e. in .DELTA.F508, the bases CTT are deleted such that ATC TTT is presented as ATT. We have determined that in constructing a target for the regional mutation to be synthesized onto the surface activated organic polymer, it is preferred that the complement to the regional mutation along the target should be located so as to maximize the number of possible mis-matches when the mutation is present. For example, if the regional mutation is located along the target distal to the aminated polypropylene, then the corresponding hybridizations of, in this example, Exon 10,.DELTA.F508 and Exon 10,Normal, are as follows: (the underlined portion is the complement to the regional mutation): ##STR11## Thus, focusing on Exon 10,.DELTA.F508, along the 16-mer Target A70, when Exon 10,.DELTA.F508 hybridizes thereto, there will be 13 complementary bases, and 3 mis-matches (indicated in brackets). By shifting the complement to the regional mutation toward the polymer, the number of mis-matches increases: ##STR12## This shift decreases the number of complementary bases on a Target A61-Exon 10,.DELTA.F508 hybridization to 10, and increases the number of mis-matches to 6, i.e. a 100% increase in mis-matches.

Detailed Description Text (164) :

For the analysis of the Exon 10,.DELTA.F508 and Exon 10,Normal amplicons, Target A61 was utilized; Target A61 was synthesized directly onto aminated polypropylene as described above. Hybridization and dehybridization conditions were as set forth in Example III. Results are presented in FIG. 4.

Detailed Description Text (168) :

The following peptides were synthesized directly onto separate aminated polypropylene membranes. These hen-egg lysozyme peptides had the following sequences:

Detailed Description Text (170) :

HEL 106-116 (SEQ. ID. NO. 13): ##STR14## The carboxyl-group of Leu (HEL 11-25) and Lys (HEL 106-116) were coupled directly to the aminated polypropylene (i.e. Leu and Lys, respectively, were the initiator biomonomers). The peptide synthesis was conducted in accordance with the general protocol described in D. Hudson; J. Org. Chem. 53:617 (1988); Milligen Technical Note 4-30, (1987). Fmoc-protected amino acids were obtained from Beckman Instruments, Inc. (Prod. Nos. Fmoc Cys (Trt): 266366; Fmoc Lys (TBOc): 266387; Fmoc Asn: 266351; Fmoc Trp: 266408; Fmoc Ala: 266342; Fmoc Val: 266414; Fmoc Leu: 266384; Fmoc Ser (OTBu): 266402; Fmoc Tyr (OTBu): 266410; Fmoc Gly: 266375; Fmoc Asp (OTBu): 266354; Fmoc His (Trt): 266377; Fmoc Met: 266390; Fmoc Arg (Mtr) was obtained from Milligen (Prod. No. 911014). Coupling reagent (1,3-di-isopropyl carbodiimide) was obtained from Aldrich (Prod. No. D12,540-7). Coupling agent (hydroxybenzotriazole) was obtained from Aldrich (Prod. No. 15,726-0). Fmoc-deblocking group (piperidine) was obtained from Aldrich (Prod. No. 10,409-4). Side-chain groups were removed using 19 ml trifluoro acetic acid (95%) (Aldrich, Prod. No. 29,953-7), 0.5 ml anisole (Aldrich, Prod. No. 12,322-6) and 0.5 ml ethyl methyl sulfide (Aldrich, Prod. No. 23,831-7), which were added to the mixture and left at room temperature for six hours, followed by washing with ether.

Detailed Description Text (171) :

To validate the presence of these particular peptides, murine anti-HEL-11-25 monoclonal antibody and murine anti HEL-106-116 monoclonal antibody were utilized in an ELISA format. Antibodies were graciously provided by Dr. Clifford Olson, Beckman Instruments, Inc. These antibodies do not cross-react with these peptides. Three conditions were analyzed: A - aminated polypropylene membrane comprising the HEL-11-25 peptide directly synthesized thereon; B - aminated polypropylene membrane comprising

the HEL-106-116 peptide directly synthesized thereon; C - control (aminated polypropylene membrane). ELISA conditions were as follows: the membranes were placed into six individual wells of a 96-well titer plate; two wells comprised membrane/HEL-11-25; two wells comprised membrane/HEL-106-116; and two wells comprised membrane. The following conditions were utilized for each well. A solution of 1% BSA was added to each well, followed by room temperature incubation for 1 hr. This was followed by 3.times.250 .mu.l washings with phosphate-buffered saline ("PBS"). The anti-HEL-11-16 was added to one well from each set and the anti-HEL-106-116 was added to one well from the remaining set. Thereafter, 100 .mu.l of goat-anti-mouse antibody conjugated with alkaline phosphatase (1:5000 dilution) (High Clone, Utah, Part #EA 1055-X) was added to each well. This was followed by room temperature incubation for 30 min; thereafter, 3.times.250 .mu.l PBS washings were conducted. Afterwards, 100 .mu.l of NBT-BCIP (nitro blue tetrazolium -Sigma N6876; 5-bromo-4-chloro-3-indolyl-phosphate- Sigma, B6149 prepared as follows: 66 .mu.l of NBT stock (0.5 g of NBT in 10 ml of dimethylformamide) and 33 .mu.l of BCIP stock (0.5 g of BCIP in 10 ml of 70% dimethylformamide) added to 10 ml of alkaline phosphatase buffer (100 mm NaCl; 5 mm MgCl<sub>2</sub>; 100 mm tris-hydroxymethyl aminomethane, pH 9.5)) solution was added to each well. A 10 min period for color (blue) development was allotted. Thereafter, the wells were washed with deionized water. The presence of blue color following the washing indicated the presence of goat-anti mouse bound to the monoclonal antibodies (results not presented).

Detailed Description Text (172):

The respective antibodies bound specifically to their respective peptides. E.g., no blue color was observed in the wells comprising membrane/HEL-11-16 and aminated polypropylene to which was added anti-HEL-106-116. No color in the aminated polypropylene indicates that non-specific binding of either antibody did not occur. The specific binding of the antibodies to specific peptides indicates, inter alia, that the HEL-11-25 and HEL-106-116 peptides were indeed correctly synthesized onto the aminated polypropylene.

Detailed Description Text (175):

Aminated polypropylene film having SAM 125 covalently attached thereto was soaked for 10 min in Hybridization Buffer. Thereafter, 3 .mu.l 615 Fluorescent Probe in 97 .mu.l Hybridization Buffer (100 pmol/100 .mu.l) was added thereto, followed by incubation for 90 min. This was followed by 4.times.200 .mu.l washings with Hybridization Buffer. The film was then removed and placed onto a glass slide, followed by analysis using the CCD camera; a laser-printer reproduction of the results are presented in FIG. 5

CLAIMS:

1. A device comprising biopolymers covalently attached to a polypropylene surface via covalent attachment sites, each of said biopolymers consisting of a known sequence of known biomonomers, and said covalent attachment sites having a structure selected from the group consisting of ##STR15##
4. The device of claim 2 wherein said biopolymers are attached to said surface at known locations.
5. The device of claim 4 wherein said biopolymers are different, each of said different biopolymer being attached at a different known location.